Part #21

Patent Docket P1363R1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Deborah Ann Ansaldi Serial No.: 09/320,100 Filed: 26 MAY 1999 For: SEPARATION OF POLYPEPTIDE MONOMERS	Group Art Unit: 1642 Examiner: J. Hunt CERTIFICATE OF FACSIMILE TRANSMISSION March 29, 2002 Date of Transmission I hereby certify that this correspondence, consisting of Amendment After Final Rejection and Amendment Transmittal, is being facsimile transmitted to the Assistant Commissioner of Patents, Washington, D.C. 20231. Janet E. Hasak Janet E. Hasak

Considered 8/19/02 AH

DECLARATION UNDER 37 CFR §1.132

Assistant Commissioner of Patents
Washington, D.C. 20231

I, Steven M. Cramer, PhD, do hereby declare and say as follows:

1. I am the same Dr. Cramer who provided a Declaration in connection with the above application filed September 20, 2001. My credentials are indicated in that Declaration and Appendix A attached to it.

2. In addition to the papers that I indicated I have reviewed in my previous Declaration, I have also reviewed the Office Action after Final Rejection dated 01/02/02. The Examiner indicates that my previous Declaration appears to be drawn to methods of purifying protein monomers from solutions consisting of multimers and/or dimers of the monomer, whereas the claims of the above application are drawn to methods of separating monomers from mixtures comprising dimers and/or multimers, but which also may contain other proteins, multimers, dimers, variants, and so forth. The Examiner further states that the high levels of yield and purity cited by me as unexpected are not commensurate in scope with the claims, asserting that the instant application only discloses these results when specific reaction conditions are used for protein purification of

homogeneous commercial solutions, and that my conclusions are drawn specifically to methods of purifying homogeneous mixtures. I hereby clarify my position.

3. My previous Declaration was not drawn to methods of purifying protein monomers from solutions consisting only of multimers and/or dimers of the monomer. My arguments apply to the claims of the application, which recite a method of separation of monomers from mixtures consisting essentially of such monomers along with their dimers and/or multimers, that is, such mixtures also may contain other components that do not materially affect the fundamental character (the basic and novel characteristics) of the invention. The instant application discloses the unexpected results of purity and yield when specific reaction conditions are used for protein purification of commercial solutions as well as anti-IgE antibody, which may contain other proteins, under section B, pages 9-10, besides the multimers and/or dimers of the monomer. See Figs. 1-6, which represent chromatograms that often show other peaks besides the monomer and dimer. My conclusions are not drawn specifically to method of purifying homogeneous mixtures. The specifics are set forth below.

4. The claimed invention is directed to a method for purifying polypeptide monomers from a mixture consisting esentially of said polypeptide monomers, and dimers or multimers of said polypeptide monomers or both dimers and multimers of said polypeptide monomers, wherein the method consists essentially of applying the mixture to a cation-exchange or anion-exchange chromatography resin in a buffer, wherein if the resin is cation-exchange, the pH of the buffer is about 4-7, and wherein if the resin is anion-exchange, the pH of the buffer is about 6-9, and eluting the mixture at a gradient of about 0-1 M of an elution salt. The purification method necessarily results in the separation of the monomer from its dimers and/or multimers present in the mixture and to such a degree that the monomer has a purity of greater than 99.5% and the monomer yield is greater than 90%.

5. At the relevant time of filing the above application (June 1, 1998), the disclosure of Yang *et al.* would not have conveyed to the skilled practitioner in this field that monomeric proteins can be separated from dimeric and/or multimeric forms thereof contained in mixtures consisting essentially of such monomers, dimers, and multimers and obtained

in a yield of such high degree utilizing the ion-exchange technique of Yang *et al.* One skilled in the chromatographic field would view Yang *et al.* in the context in which it is written. Ion-exchange chromatography is a common method for separating proteins, and Yang *et al.* merely utilizes this technique to carry out what would be expected in the art. Thus, Yang *et al.* are separating polypeptide monomers from other monomeric forms thereof (such as differently glycosylated or post-translationally different immunoglobulins), or from totally different polypeptide monomers contained in the ascites and sera, or from dimers and/or multimers that may be naturally contained in ascites and sera. However, Yang *et al.* do not explicitly disclose separation of such monomers from their own dimers and/or multimers contained in a mixture consisting essentially of these two or three components. The skilled artisan would not have believed as of June 1, 1998 that separation of monomers from their own dimers and/or multimers (in a mixture optionally containing other materials, provided they do not materially change the fundamental and novel character of the process) to produce therapeutically acceptable polypeptides could be accomplished at such high yield and purity by ion-exchange chromatography. Before the filing date of this application, the skilled chromatographic separation scientist was using size-exclusion chromatography for this purpose. Thus, in my view one of reasonable skill in the field would not believe that Yang *et al.* discloses all stated features and elements of the claimed invention.

6. The protein loads utilized by Yang *et al.* in their chromatography would not allow one of reasonable skill in this field to reach the conclusion that purification of monomers from their dimers and/or multimers in a mixture consisting essentially of same would be feasible, much less would necessarily flow from the disclosure of Yang *et al.*

7. Moreover, one of ordinary skill in the field as of June 1998 would not have appreciated or recognized from Yang *et al.* the feature thought to be inherent, namely, that dimers and multimers could be separated from their own monomers in a mixture that consists essentially of these components, let alone the high yields or purity levels stated. As mentioned above, there are chromatographic media designed specifically to separate proteins by size (size-exclusion chromatography) and these were used by practitioners before June 1998 to achieve the separation of monomers from their dimers and/or multimers in

mixtures consisting essentially of such monomers and dimers and/or multimers as claimed. The practitioner versed in this field would not have recognized that monomers could be separated to the level of purity and yields claimed; evidence to the contrary is shown by the fact that such ion-exchange purification methods were not used to purify monomers from their dimers and/or multimers in mixtures consisting essentially of these components before the present invention was made, but rather size-exclusion chromatography. Since Yang *et al.* do not contain the supporting data or text describing separation of monomers from their dimers and/or multimers in mixtures consisting essentially of them, the skilled practitioner, without the teachings disclosed for the first time by the present application, would not have recognized that separation of monomers from their dimers and/or multimers in mixtures consisting essentially of such monomers and dimers and/or multimers at such high yields and purity would be possible using the claimed method of purification.

8. Similarly, all of the elements and features of the claimed invention are not disclosed by Hahn *et al.*, since the claims require that the monomer be separated from its own dimers and/or multimers in mixtures consisting essentially thereof. Hahn *et al.* teach separation of various different proteins from each other, all of which are contained in bovine whey, such as IgG from lactoferrin and from lactoperoxidase (see, e.g., Table 1 on page 280). There is no evidence in Hahn *et al.* that any separation has occurred between the monomer and any of its own dimers and/or multimers present in the mixture consisting essentially of them, as required by the present claims, as opposed to dimers and/or multimers that may naturally be present in bovine whey.

9. The Examiner states that Hahn *et al.* teach purification of immunoglobulins using an identical method to that instantly claimed and thus purification would include elution of the IgG monomers from a mixture (bovine whey) that contains monomers and dimers or multimers. However, as established above, the separation of monomers from dimers and/or multimers thereof in a mixture consisting essentially of them (the characteristic of the claimed invention deemed to be inherent) is not necessarily or actually achieved by practicing the ion-exchange technique with the protein load mixture used by Hahn *et al.* to purify immunoglobulins from bovine whey. The ordinarily skilled scientist versed in purification techniques would not have

reasonably concluded from the teachings of Hahn *et al.* that purification of monomers from their dimers and/or multimers contained in mixtures consisting essentially of these two or three components would be feasible at such high levels of yield and purity in June 1998.

10. Further, the skilled scientist in chromatography would not have appreciated or recognized as of June 1998 from Hahn *et al.* that monomers could be separated from their dimers and/or multimers contained in mixtures consisting essentially of the monomers, dimers, and/or multimers. Since Hahn *et al.* do not contain the requisite teachings, the skilled scientist would not have recognized that separation of monomers from their dimers and/or multimers in mixtures consisting essentially thereof would be possible at such high levels of yield and purity using the claimed method of purification.

11. The claimed invention would also not have been obvious as of June 1998 from the combination of Tayot *et al.* with Yang *et al.* Yang *et al.* is discussed above. Tayot *et al.* does not disclose or suggest how one skilled in the art might separate monomers from their own dimers and/or multimers contained in mixtures that consist essentially of such monomers and dimers and/or multimers. Instead, hemoglobin, gamma-globulins, and albumin are separated from each other and presumably also from other unrelated proteins in the blood (see, e.g., claim 1), or hemoglobin and albumin are separated from each other and presumably also from other unrelated proteins in the blood (see, e.g., claim 10). These protein moieties are not related as monomers and dimers of such monomers and/or multimers of such monomers, as is required in the claimed purification method of the above application. The anion-exchange step described in Tayot *et al.* is designed such that only albumin binds to the column and the hemoglobin and immunoglobulins flow through the column. The gamma-globulins are separated from the hemoglobin by precipitation in ice-cold ethanol. Therefore, the purification of IgGs is achieved by a method (precipitation) completely distinct from the present claimed ion-exchange method (see col. 4, lines 10-54 of Tayot *et al.*). Tayot *et al.* further state that the Ig precipitate "...must then be subjected to other purification operations already known so as to prepare immunoglobulins which may be used in human therapeutics" (col. 4, lines 48-51), just as with albumin (compare col. 4, lines 29-31). It is evident that no mention is made of the purification of gammaglobulins or albumin from its dimers and/or multimers.

These statements regarding further purification that is required actually teach away from the claims of the above application where no further purification step is used. Hence, one versed in this art, in my opinion, would not be motivated to combine the disclosure of Yang *et al.* with that of Tayot *et al.*

12. The claimed invention also would not have been obvious from the Oncogene Science catalog along with Yang *et al.* and/or Hahn *et al.* The latter references contain no details or directions to instruct the skilled artisan on how to obtain pure antibodies from impure mixtures consisting essentially of dimers and multimers of the antibody monomers to be purified for the reasons noted above. As to the former reference, I did not set forth in my previous Declaration that the Oncogene Science antibodies are already purified; instead, I indicated that the antibodies of the catalog are actually only research-grade material, so that their level of purity has no bearing on the level of purity needed to obtain antibodies suitable for therapeutic needs, as the claimed level of greater than 99.5% reflects. The catalog does not provide the motivation to or desirability of obtaining further purified antibodies, since it is offering for sale less purified antibodies that presumably need no further purification (they are not antibodies that would be used in therapeutics, which require a high level of purification as set forth in claim 1). Hence, the catalog would teach away from the invention by indicating no need for further purification of the antibodies. Neither Yang *et al.* nor Hahn *et al.* nor the Oncogene Science catalog even acknowledges the existence of dimers and/or multimers of polypeptide monomers, let alone that a separation thereof from the monomers in mixtures that may contain other components can occur so as to obtain highly pure monomeric antibodies. The combined references would not have suggested the claimed invention as set forth above, particularly with the purity and yield results.

13. Furthermore, when I first heard about the invention claimed in the above application, I was surprised that the technique could be used to purify monomers from their own dimers and/or multimers (which are all contained in a mixture consisting essentially of such components) at such unexpectedly high minimum purity and yield levels obtained as claimed, i.e., greater than 99.5% and greater than 90%, respectively. Size-exclusion chromatography was the gold standard at the time for distinguishing between these very similar protein species. My colleagues and I working in the separation arts would not have

expected from Yang *et al.* combined with Tayot *et al.* or from Yang *et al.* and Hahn *et al.* in combination with the selected catalog pages that such a high yield and purity could be achieved.

14. In summary, the above citations alone or in combination merely disclose that proteins can be purified to some degree using ion-exchange chromatography. In particular, the disclosures clearly show separation of IgG from BSA or IgG partially separated from whey, serum, or ascites proteins, etc. None of the cited references even mentions the existence of dimers and/or multimers of polypeptide monomers. Nowhere do these references, alone or in combination, mention or suggest the purification of monomers from their dimers/multimers in mixtures consisting essentially of the monomers and dimers and/or multimers using ion-exchange chromatography as claimed, much less with the claimed yield and purity results. Such results would not have necessarily followed from practicing the teachings of these references due to the nature of the mixtures being loaded on the column in these references, and the skilled practitioner would not have appreciated or expected from these teachings that such could be done.

15. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

3/27/02
Date

Steven M. Cramer, Ph.D.